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Nucleotide Sequences of the *sfuA*, *sfuB*, and *sfuC* Genes of *Serratia marcescens* Suggest a Periplasmic-Binding-Protein-Dependent Iron Transport Mechanism

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The cloned *sfu* region of the *Serratia marcescens* chromosome confers the ability to grow on iron-limited media to an *Escherichia coli* K-12 strain that is unable to synthesize a siderophore. This DNA fragment was sequenced and found to contain three genes termed *sfuA*, *sfuB*, and *sfuC*, arranged and transcribed in that order. The *sfuA* gene encoded a periplasmic polypeptide with calculated molecular weights of 36,154 for the precursor and 33,490 for the mature protein. The *sfuB* gene product was a very hydrophobic protein with a molecular weight of 56,589. The *sfuC* gene was found to encode a rather polar but membrane-bound protein with a molecular weight of 36,671 which exhibited strong homology to consensus sequences of nucleotide-binding proteins. The number, structural characteristics, and locations of the SfuABC proteins were typical of a periplasmic-binding-protein-dependent transport mechanism. How Fe^{3+} is solubilized and taken up across the outer membrane remains an enigma.

The insolubility of Fe^{3+} at neutral pH requires the formation of complex compounds with siderophores for this ion to be taken up by bacteria and fungi. The Fe^{3+} siderophores are first bound to highly specific receptor proteins in the outer membranes of *Escherichia coli* and other gram-negative bacteria. Subsequently, they are taken up in an energy- and TonB-dependent step across the outer membrane into the periplasmic space. Mutations in the *exbB* and *exbD* genes (6) result in a strongly reduced rate of iron uptake (8), implicating the products of these genes in the uptake process.

Proteins required for the transport of Fe^{3+} via ferrichrome, coprogen, ferrioxamine B, rhodotorulic acid, and aerobactin (11), as well as citrate (23) and enterochelin (14, 15), are found in the periplasmic space. In addition, one (10, 11) or two (23) very hydrophobic proteins are localized in the cytoplasmic membrane. Furthermore, a rather hydrophilic but membrane-associated protein with sequence regions typical of nucleotide-binding proteins has been identified (2, 4, 23). The structural features and the locations of the proteins are similar to those of periplasmic-binding-protein (PBP)-dependent transport systems, such as those that have been characterized for certain amino acids and sugars (1). Therefore, it has been proposed that the uptake of Fe^{3+} siderophores follows the mechanism of PBP-dependent systems (2, 4, 11, 14, 15, 23). Transport across the outer membrane is considered to occur independently of transport across the cytoplasmic membrane (21).

Recently, we cloned a 4.8-kilobase DNA fragment from *Serratia marcescens* which conferred on an *E. coli* mutant unable to produce enterochelin, its own siderophore, the ability to grow on a low-iron medium (27). Surprisingly, the uptake of iron was independent of *tonB* and *exbB*, and neither a siderophore nor an outer membrane protein could be related to this transport system. Four proteins with molecular masses of 40, 38, 36, and 34 kilodaltons (kDa) were identified. The 40-kDa protein was a precursor of the 38- and 34-kDa proteins. In this paper, we report the nucleotide sequences of three genes and their products which determine this unusual iron transport system.

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MATERIALS AND METHODS

Bacterial strains and media. *E. coli* K-12 H1443 *aroB araD lac thi rpsL* was grown in tryptone-yeast extract medium or in iron-deficient nutrient broth containing 0.2 mM 2,2′-dipyridyl as described previously (27).

DNA sequencing. DNA fragments of plasmid pSZ1 (27) were excised with the restriction enzymes *Sal*I, *Pst*I, *Cla*I, *Hin*dIII, *Bgl*II, *Mlu*I, *Bbe*I, *Bam*HI, *Taq*I, *Hin*dII, *Nae*I, *Sau*3A, and *Hpa*II and cloned into the appropriate sites of bacteriophages M13mp18 and M13mp19 for sequencing by the enzymatic dideoxy-chain termination method (20, 26). [α-^{35}S]dATP was employed for labeling, and dGTP was replaced by 7-deaza-dGTP for sequencing regions which showed band compression on sequencing gels. The sequencing kits used contained Klenow polymerase and T7-derived Sequenase and were from Pharmacia (Uppsala, Sweden) and United States Biochemical Corp. (Cleveland, Ohio), respectively. Sequence data were analyzed with the Pustell Sequence Analysis Program (International Biotechnologies, Inc., New Haven, Conn.) and with GenBank (release 55; IntelliGenetics, Los Alamos, N.M.).

Site-specific mutagenesis. Cytosine at base pair (bp) 3799 was replaced with adenine by the gapped duplex DNA method (12) with a site-directed mutagenesis kit (catalog no. 1027 492; Boehringer GmbH, Mannheim, Federal Republic of Germany). The *Bgl*II-*Hin*dIII fragment of the pSZ1 insert was cloned into phage M13mp9 double-stranded DNA. A synthetic primer from bp 3790 to 3812 of the nucleotide sequence (see Fig. 2) containing the exchanged nucleotide was used as a mutagen. The mutation was verified by sequencing. The mutated DNA fragment was recloned and sequenced again to confirm that the strain whose growth on low-iron medium was unaffected carried the mutated plasmid.

Identification of plasmid-encoded proteins and isolation of subcellular fractions. The procedures used were recently described (11). Phenylmethylsulfonyl fluoride (3 mM) and

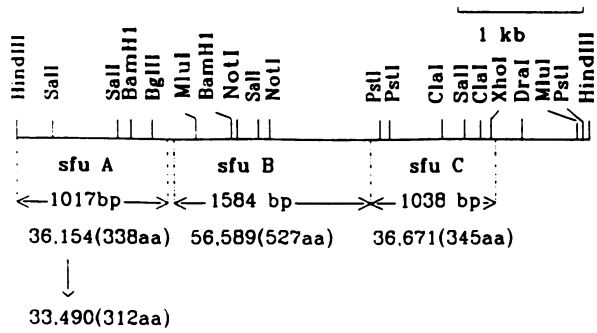


FIG. 1. Physical map of the DNA fragment on plasmid pSZ1 containing the *sfu* structural genes of *S. marcescens*. The genes are transcribed from left to right. Cleavage sites of selected restriction endonucleases are indicated. The molecular weights of the proteins and the number of amino acids (aa) encoded by the open reading frames indicated by horizontal arrows are shown. For *sfuA*, the molecular weights of the precursor and the mature protein are given. kb, Kilobase.

p-aminobenzamidine (5 mM) were added to cell envelopes before treatment with Triton X-100–$MgCl_2$ to prevent proteolytic degradation. In vitro protein synthesis was studied with the procaryotic DNA-directed translation kit (N.380Y/Z) of Amersham Buchler, Brunswick, Federal Republic of Germany.

RESULTS AND DISCUSSION

Nucleotide sequences of the *sfuA*, *sfuB*, and *sfuC* genes. Plasmid pSZ1 contains a 4.8-kilobase *Hin*dIII fragment of the *S. marcescens* chromosome cloned into the *Hin*dIII site of plasmid pBR322 (27). *E. coli* H1443 *aroB* transformed with pSZ1 grows on nutrient broth made iron deficient by the addition of 0.2 mM dipyridyl (27). The gene encoding the related 40-, 38-, and 34-kDa proteins was contained in a 1.5-kilobase *Hin*dIII-*Sal*I fragment to the left of the insert (Fig. 1). The gene encoding the 36-kDa protein was localized in the right *Bam*HI-*Eco*RI fragment (the *Eco*RI site of the vector). No protein could be identified between these two regions, although Tn*1000* insertions close to the right *Bam*HI site of insert (Fig. 1) abolished growth of H1443 transformants on iron-limited medium even though expression of the downstream *sfuC* gene was not inhibited (27).

Both DNA strands of the insert shown in Fig. 1 were completely sequenced. Three open reading frames, designated *sfuA*, *sfuB*, and *sfuC*, were found in the transcription polarity of the iron transport genes previously determined (27). *sfuA* starts at nucleotide 168 and ends at nucleotide 1184 (Fig. 2). It is preceded by a ribosome-binding site 7 nucleotides from the ATG codon which corresponds in 5 of 8 nucleotides with the consensus sequence derived from *E. coli* genes (22). Possible promoter sequences are located at bp 23 to 80 and bp 92 to 143; the latter sequences exhibit a somewhat better homology to the −10 and −35 consensus sequences (19). The sequence between nucleotides 54 and 72, 5′-ttTAATacgAATCgTTTTC-3′, corresponds in 13 (nucleotides in capital letters) of 19 bases with the Fur iron repressor-binding site 5′GAT(A,T)ATGAT(A,T)AT(C,T)ATTTTC-3′ (3, 5, 18). A similar sequence, GATTGTCAT AATTTCCCC, was found upstream of the *S. marcescens* hemolysin genes (17), and it was shown that their transcription was controlled by iron via the Fur repressor in *E. coli* and by iron in *S. marcescens* (16).

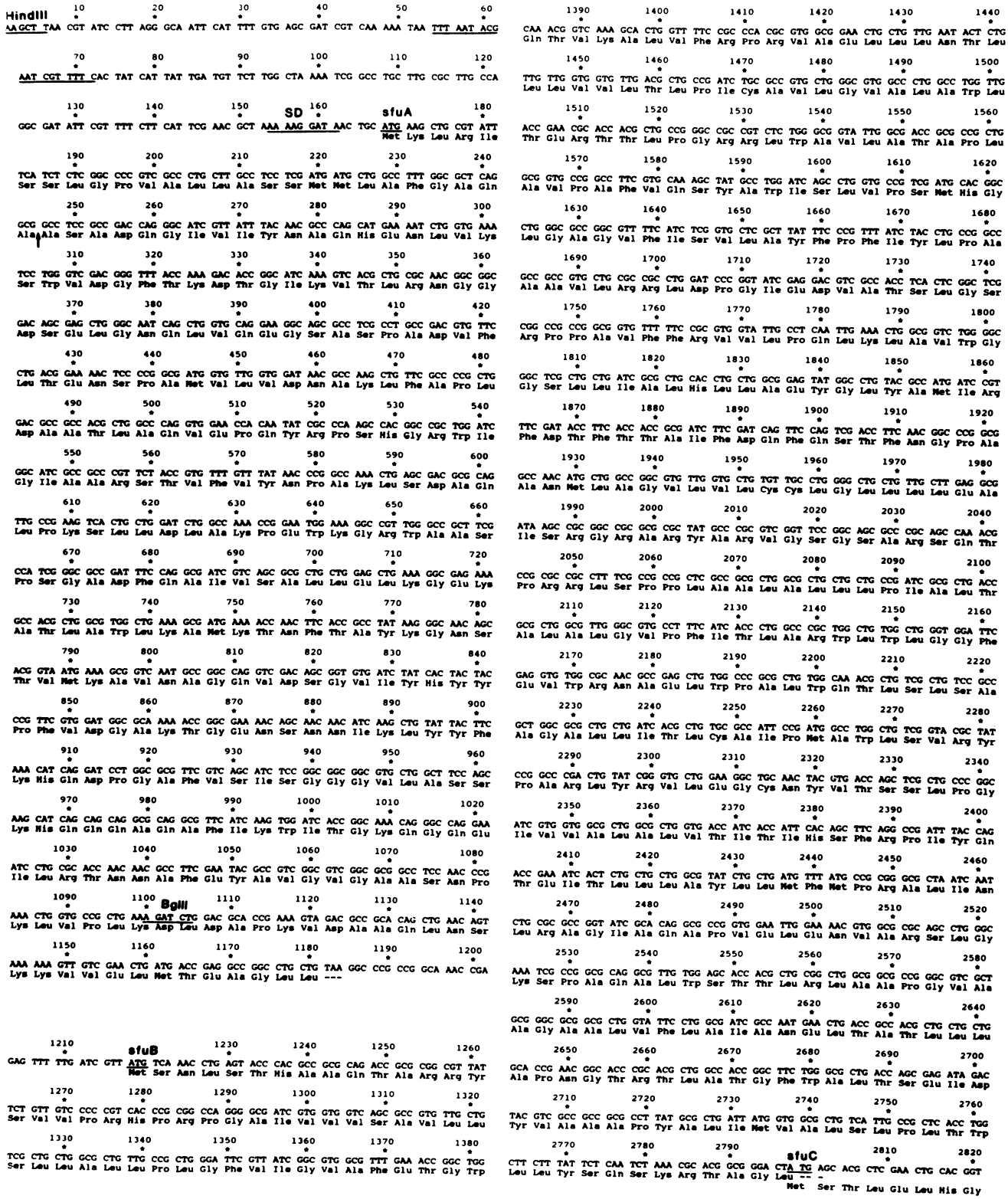
The *sfuB* gene starts 33 bp after the *sfuA* gene and comprises 1,584 bp (Fig. 1 and 2). No obvious transcription initiation and termination sequences could be discerned. There is no sequence upstream of the ATG start codon complementary to the 16S rRNA which could serve as a ribosome-binding site. In fact, the level of synthesis of SfuB is very low (see below); this low level of synthesis seems to be caused by a weak ribosome-binding site.

The start codon of *sfuC* overlaps in two bases with the stop codon of *sfuB*. Although *sfuC* was transcribed from pSZ1 when transposon Tn*1000* was inserted upstream of *sfuC* or when *sfuC* alone was cloned in pBR322, the homology of possible promoter regions with the consensus sequences was very low. It is possible that in these cases, transcription of *sfuC* started within the transposon and the vector, respectively. A palindromic sequence composed of 2 AT pairs followed by 7 GC pairs in the stem and 7 unpaired bases in the loop is located downstream of *sfuC* (Fig. 2). Such a secondary structure is typical for a Rho-independent transcription termination site (19). The nucleotide sequence of the *sfu* locus suggests that the three genes form an operon in which transcription starts upstream of *sfuA* and terminates downstream of *sfuC*. There is no obvious ribosome-binding site upstream of *sfuC*. The small amount of SfuC, compared with SfuA (Fig. 3), is probably the result of a lower translation initiation frequency.

An open reading frame (bp 3968 to 4492) with a promoter encoding a 19-kDa protein is located downstream of *sfuC*. DNA constituting the open reading frame can be deleted by *Dra*I digestion without affecting growth on nutrient broth-dipyridyl agar plates. Strain H1443 containing the *Hin*dIII-*Dra*I fragment of the pSZ1 insert cloned into the pT7-5 vector (cleaved with *Hin*dII-*Sma*I) grew as well as H1443 carrying pSZ1 did.

The complementary strand of the insert of pSZ1 contains two large open reading frames extending from bp 4583 to 3869 and from bp 3820 to 2774. However, no labeled protein was detected in cells transformed with the *Hin*dIII insert of pSZ1 cloned downstream of the phage T7 gene 10 promoter (pT7-6) (27). In contrast, cells containing the same fragment cloned with the opposite transcriptional polarity into pT7-5 synthesized the 40-, 38-, 36-, and 34-kDa proteins (27). The open reading frame starting immediately after the right *Hin*dIII site (Fig. 1) contains no translation start codon (Fig. 2). The autoradiogram of [^{35}S]methionine-labeled cells transformed with the *Bam*HI-*Eco*RI fragment revealed a 30-kDa protein (data not shown) which was probably translated from an ATG start codon within the vector, giving rise to a calculated 27-kDa protein (239 amino acids from the insert plus 6 amino acids encoded by the vector DNA). The second open reading frame is contained within *sfuC* and comprises 1,047 bp with an ATG start codon, a sequence likely to be a promoter, and a ribosome-binding site. Although no polypeptide was observed in cells carrying this region downstream of the T7 promoter, a weak expression below the detection limit could be sufficient to contribute to iron transport. Therefore, a stop codon was introduced close to the start of the open reading frame by converting a C at bp 3799 to an A (GAG to TAG on the complementary strand), which left the amino acid sequence of the SfuC protein unaltered (CTA instead of CTC). The pSZ1 derivative with this altered nucleotide sequence supported growth of strain H1443 on low-iron medium, which indicates that synthesis of this protein was not required for iron transport.

Amino acid sequences and subcellular localization of the Sfu proteins. (i) Periplasmic SfuA protein. SfuA is composed of 338 amino acids and contains a typical signal sequence found


HindIII
SD
sfuA
BglII
sfuB
sfuC

```
        2830          2840          2850          2860          2870          2880
         *             *             *             *             *             *
ATC GGA AAA TCT TAC AAC GCC ATC AGA GTG CTG GAA CAC ATC GAC CTG CAG GTT GCC GCC
Ile Gly Lys Ser Tyr Asn Ala Ile Arg Val Leu Glu His Ile Asp Leu Gln Val Ala Ala

        2890          2900          2910          2920          2930          2940
         *             *             *             *             *             *
GGC AGC CGC ACG GCG ATC GTC GGC CCT TCC GGC TCC GGC AAA ACC ACT CTG CTG CGC ATC
Gly Ser Arg Thr Ala Ile Val Gly Pro Ser Gly Ser Gly Lys Thr Thr Leu Leu Arg Ile

        2950          2960          2970          2980          2990          3000
         *             *             *             *             *             *
ATC GCC GGC TTT GAA ATC CCC GAC GGC GGC CAG ATC CTG CTG CAG GGA CAA GCC ATG GGC
Ile Ala Gly Phe Glu Ile Pro Asp Gly Gly Gln Ile Leu Leu Gln Gly Gln Ala Met Gly

        3010          3020          3030          3040          3050          3060
         *             *             *             *             *             *
AAC GGC AGC GGC TGG GTG CCT GCG CAT CTG CGC GGC ATC GGT TTC GTT CCG CAG GAT GGC
Asn Gly Ser Gly Trp Val Pro Ala His Leu Arg Gly Ile Gly Phe Val Pro Gln Asp Gly

        3070          3080          3090          3100          3110          3120
         *             *             *             *             *             *
GCG TTG TTC CCG CAC TTT ACC GTC GCC GGC AAC ATC GGT TTT GGC CTC AAA GGC GGC AAG
Ala Leu Phe Pro His Phe Thr Val Ala Gly Asn Ile Gly Phe Gly Leu Lys Gly Gly Lys

        3130          3140          3150          3160          3170          3180
         *             *             *             *             *             *
CGC GAG AAA CAG CGG CGC ATC GAG GCG CTG ATG GAG ATG GTG GCG CTG GAT CGC CGT CTG
Arg Glu Lys Gln Arg Arg Ile Glu Ala Leu Met Glu Met Val Ala Leu Asp Arg Arg Leu

        3190          3200          3210          3220          3230          3240
         *             *             *             *             *             *
GCG GCG CTG TGG CCG CAC GAG TTG TCC GGC GGG CAG CAA CAG CGG GTC GCG CTG GCG CGC
Ala Ala Leu Trp Pro His Glu Leu Ser Gly Gly Gln Gln Gln Arg Val Ala Leu Ala Arg

        3250          3260          3270          3280          3290          3300
         *             *             *             *             *             *
GCC CTG TCG CAG CAA CCC CGG CTG ATG CTG CTG GAT GAA CCG TTC TCG GCG CTG GAT ACC
Ala Leu Ser Gln Gln Pro Arg Leu Met Leu Leu Asp Glu Pro Phe Ser Ala Leu Asp Thr

        3310          3320          3330          3340          3350          3360
         *             *             *             *             *             *
GGC CTG CGC GCC GCC ACC CGC AAA GCG GTG GCC GAA CTG CTG ACG GAG GCC AAG GTG GCA
Gly Leu Arg Ala Ala Thr Arg Lys Ala Val Ala Glu Leu Leu Thr Glu Ala Lys Val Ala

        3370          3380          3390          3400          3410          3420
         *             *             *             *             *             *
TCG ATT CTG GTC ACC CAC GAT CAG AGC GAG GCG CTG TCG TTC GCC GAT CAG GTG GCG GTG
Ser Ile Leu Val Thr His Asp Gln Ser Glu Ala Leu Ser Phe Ala Asp Gln Val Ala Val

        3430          3440          3450          3460          3470          3480
         *             *             *             *             *             *
ATG CGC AGC GGC CGG CTG GCG CAG GTG GGC GCG CCG CAG GAT CTC TAT CTG CGG CCG GTT
Met Arg Ser Gly Arg Leu Ala Gln Val Gly Ala Pro Gln Asp Leu Tyr Leu Arg Pro Val

        3490          3500          3510          3520          3530          3540
         *             *             *             *             *             *
GAT GAG CCG ACC GCC AGC TTC CTT GGC GAA ACG CTG GTG CTG ACC GCC GAA CTG GCG CAC
Asp Glu Pro Thr Ala Ser Phe Leu Gly Glu Thr Leu Val Leu Thr Ala Glu Leu Ala His

        3550          3560          3570          3580          3590          3600
         *             *             *             *             *             *
GGC TGG GCC GAC TGC GCA CTG GGG CGC ATC GCC GTC GAC GAT CGC CAA CGC AGC GGC CCG
Gly Trp Ala Asp Cys Ala Leu Gly Arg Ile Ala Val Asp Asp Arg Gln Arg Ser Gly Pro

        3610          3620          3630          3640          3650          3660
         *             *             *             *             *             *
GCG CGC ATC ATG CTG CGC CCG GAG CAG ATT CAA ATC GGT TTG TCC GAT CCG GCG CAG CGC
Ala Arg Ile Met Leu Arg Pro Glu Gln Ile Gln Ile Gly Leu Ser Asp Pro Ala Gln Arg

        3670          3680          3690          3700          3710          3720
         *             *             *             *             *             *
GGC CAG GCG GTG ATC ACC GGC ATC GAT TTC GCC GGC TTC GTC TCC ACC CTC AAT CTG CAA
Gly Gln Ala Val Ile Thr Gly Ile Asp Phe Ala Gly Phe Val Ser Thr Leu Asn Leu Gln

        3730          3740          3750          3760          3770          3780
         *             *             *             *             *             *
ATG GCG GCC ACC GGG GCA CAG CTC GAG ATC AAA ACC GTC AGC CGC GAA GGC CTG CGG CCC
Met Ala Ala Thr Gly Ala Gln Leu Glu Ile Lys Thr Val Ser Arg Glu Gly Leu Arg Pro

        3790          3800          3810          3820          3830          3840
         *             *             *             *             *             *
GGT GCT CAG GTC ACT CTC AAC GTG ATG GGC CAG GCG CAT ATT TTC GCC GGC TGA CTC TCT
Gly Ala Gln Val Thr Leu Asn Val Met Gly Gln Ala His Ile Phe Ala Gly ---
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        3850          3860          3870          3880          3890          3900
         *             *             *             *             *             *
TAA GGC CGG GCT GCC CCC CCG GCC TTC TCA GCG GCA AAT CTT GTC TCG GCG CGG CTT TTT

        3910          3920          3930          3940          3950          3960
         *             *             *             *             *             *
CGG CGC CAG CAG ATA ATC CAC CAC GCT TTC GTC AAC GCA GCT GTC GAT ACC GTT TAG CGC

        3970          3980          3990          4000          4010          4020
         *             *             *             *             *             *
CAG CGT ATG CCC GTC GCG TTC GCG GGT GAT CAG CGG GCT TTT AAA GGC GGC GGC CAT CGC

        4030          4040          4050          4060          4070          4080
         *             *             *             *             *             *
TTG GGC ATT GCG GTA CGG CGT GGT CGG ATC GTA GCG CTG CGC GAC GAA CAG CAG CGG CGG

        4090          4100          4110          4120          4130          4140
         *             *             *             *             *             *
CAG CGC GGC GGA CGC CAC CGG GGT GTG CGG CCG ATC TTT GCC GCG GTA CGG CCA CAG ATC

        4150          4160          4170          4180          4190          4200
         *             *             *             *             *             *
GCA CAT TTC CAG CGG ATA CTC GTG CAA CGG CAG GTA GTG AGC GTA CAA AGC GGC GGT ATT

        4210          4220          4230          4240          4250          4260
         *             *             *             *             *             *
GAT CTC CTG CCG CTG CCT GCG CAA CTG CTG CGG ATC GGC CGT CGG GTT GGC GAC GTC GGC

        4270          4280          4290          4300          4310          4320
         *             *             *             *             *             *
GCA GGT GAT GAC GTT CAG GGC GTC ATC GGC GTC CGG CGA ATA GCT TTC GTC GAT CAG ATC

        4330          4340          4350          4360          4370          4380
         *             *             *             *             *             *
GGA AAC CTG CTG CCC CGC GAT GCC GGC ATC GAG CTG GCG CAG CAC GGT CGC CAG TTC ATG

        4390          4400          4410          4420          4430          4440
         *             *             *             *             *             *
CCA GCG CTC CGG CCA CAG CAG CAG CGA ACG CGT GAC CGT CAG CAC ATC GTC GGC GGA GAT

        4450          4460          4470          4480          4490          4500
         *             *             *             *             *             *
TTC ATA CCC CGC GGC GGT AAC GAA CGG TTG ATC GTG CAG CTT GCG CAG TAA GGC ATG GTA

        4510          4520          4530          4540          4550          4560
         *             *             *             *             *             *
GCG CTG CAG CGC CTG ATT AGC GCC GCC GCC CAG CTG GCA GCT GTC GGT TTT GCC GCA GTA

        4570
         *
GGC GGC GAA GCG CAG GAA GCT T
          HindIII
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FIG. 2. DNA sequence of the noncoding strand of *sfuABC*. The numbering begins at the *Hin*dIII site upstream of *sfuA*. Putative Shine-Dalgarno sequences (SD) are indicated. Dashes indicate stop codons. The vertical arrow in SfuA shows the proposed cleavage site of the leader peptidase. The potential transcription termination site downstream of *sfuC* is indicated.

in exported proteins. Cleavage of the signal peptide most likely occurs between the two alanine residues at positions 26 and 27. The calculated molecular weight of the precursor is 36,154; that of the mature protein is 33,490. Previously, we found three proteins with masses of 40, 38, and 34 kDa encoded by the *sfuA* locus, and it was shown that the 40-kDa protein was a precursor of the 38- and 34-kDa proteins (27). Apparently, a portion of the primary translation product is processed further. In the *E. coli* iron-enterochelin transport system, multiple *fepB*-derived periplasmic proteins were also found (14, 15). To localize the SfuA protein within cells, transformants containing either the previously described (27) *Hin*dIII insert (pAA1) or the *Hin*dIII-*Pst*I fragment (pAA122) of pSZ1 in plasmid pT7-5 or pT7-6 (24), respectively, were labeled with [^{35}S]methionine. Cells were converted to spheroplasts, and the released content of the periplasm was electrophoretically separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and autoradiographed. A single protein with an apparent molecular mass of 35 kDa was found in the periplasmic fraction of cells expressing all three *sfu* genes (pAA1) (Fig. 3, lane 5) or only SfuA and SfuB (pAA122) (Fig. 3, lane 10). The membrane fraction of lysed spheroplasts derived from pAA1 transformants contained only a 37-kDa protein (Fig. 3, lane 2) which was absent in pAA122 transformants. No Sfu protein was in the outer and cytoplasmic membrane fraction (Fig. 3, lanes 3, 4, 8, and 9). The agreement between the determined molecular mass of 35 kDa for the periplasmic protein with

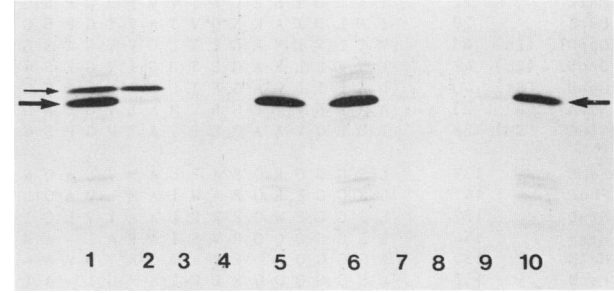

FIG. 3. Fluorogram of ^{35}S-methionine-labeled proteins of *E. coli* WM1576(pGP1-2) transformed with plasmid pAA1 *sfuABC* (lanes 1 to 5) and pAA122 *sfuAB* (lanes 6 to 10). The proteins of whole cells (lanes 1 and 6), cell envelopes (lanes 2 and 7), outer membrane fractions (lanes 3 and 8), cytoplasmic membrane fractions (lanes 4 and 9), and the periplasmic fractions (lanes 5 and 10) were separated by electrophoresis on sodium dodecyl sulfate-polyacrylamide gels. Symbols: ➡, SfuA; →, SfuC.

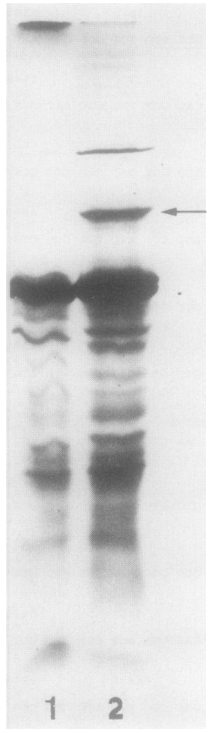

FIG. 4. Fluorogram of [^{35}S]methionine-labeled proteins synthesized in an in vitro transcription-translation system programmed by plasmid pAA9 (*sfuB*). The sample was either heated (lane 1) or maintained at room temperature (lane 2) in the sample buffer before electrophoresis in a sodium dodecyl sulfate-polyacrylamide gel. The arrow indicates the SfuB protein.

the calculated molecular mass of 34 kDa for the mature protein and the absence of the 37-kDa membrane protein (SfuB, see below) in cells containing pAA122 (encoding *sfuA* and *sfuB*) identify the 35-kDa protein as SfuA. Comparison of the amino acid sequence with over 1,200 sequences in GenBank revealed no larger homologies to known proteins. It has previously been found that periplasmic proteins, although very similar in secondary and tertiary structure, differ in primary structure (1).

(ii) Hydrophobic SfuB protein. The amino acid sequence deduced from the nucleotide sequence of *sfuB* comprises 527 amino acids, most of which are hydrophobic. The mean hydrophobicity (13) along the entire sequence is +0.82, which is about as high as the hydrophobicities of the FhuB protein (+0.965) of the *E. coli* iron-hydroxamate transport system (10, 11), the FecC (+0.85) and FecD proteins (+0.997) of the iron-citrate transport system (23), and lactose permease (+0.91) (25). The molecular weights of the membrane proteins hitherto determined for iron and the mechanistically similar vitamin B_{12} transport system were either in the range of 30,000 (FecC, 35,367; FecD, 34,148 [23]; FepC, 31,000 [14, 15]; BtuC, 31,683 [7]) or twice this weight (FhuB, 70,329 [10, 11]). FhuB exhibits homology between the N-terminal and C-terminal halves of the molecule, from which a gene duplication was deduced (10, 11). In the case of FhuB, the two homologous integral cytoplasmic membrane proteins usually found in PBP-dependent systems were obviously fused into one. SfuB, with a calculated molecular weight of 56,589, could also be the result of two fused polypeptides. Homologies were found within the polypeptide, but these were confined to a few short segments. The most obvious ones are as follows. (i) QTARRYS (reading from amino acid 10) is homologous to the start of the second half of the polypeptide (residue 274, QTPRRLS). (ii) RVAEL (reading from amino acid 66), is homologous to RVAEL at residue 319. (iii) EDVATSLGSRP (reading from

TABLE 1. Comparison of nonpolar membrane transport proteins containing sequences homologous to nucleotide-binding domains[a]

PROTEIN		RESIDUE	CONSERVED SEQUENCE
FecE		21	V S L S L P I G K I T A L I G P N G C G K S T L - L N C F S R L
FhuC		30	L S L T F P A G K V T G L I G H N G S G K S T L - L K M L G R H
BtuD		19	L S G E V R A G E I L H L V G P N G A G K S T L - L A R M A G M
HisP		25	V S L Q A R A G D V I S I I G S S G S G K S T F - L R C I N F L
MalK		22	I N L D I H E G E F V V F V G P S G C G K S T L - L R M I A G L
PstB		29	I N L D I A K N Q V T A F I G P S G C G K S T L - L R T F N K M
OppD1	(Ec)	43	V T L R L Y E G E T L G V V G E S G C G K S T F - A R A I I G L
OppD2	(Ec)	42	L N F S L R A G E T L G I V G E S G S G K S Q T - A F A L M G L
OppD	(St)	40	L N F T L R A G E T L G I V G E S G S G K S Q S R L R - L M G L
RbsA	(N)	23	A A L N V Y P G R V M A L V G E N G A G K S T M - M K V L T G I
SfuC	(SM)	22	I D L Q V A A G S R T A I V G P S G S G K T T L - L R I I A G F
FecE		139	L S G G Q R Q R A F L A M V L A Q N T P - - - - - - - V V L L D E P T T Y L D I N H Q V D L M R L M G E
FhuC		141	L S G G E R Q R A W I A M L V A Q D S - R - - - - - - - C L L L D E P T S A L D I A H Q V D V L S L V H R
BtuD		127	L S G G E W Q R V R L A A V V L Q I T P Q A N P A G Q L L L L D E P M N S L D V A Q Q S A L D K I L S A
HisP		154	L S G G Q Q Q R V S I A R A L - A M E P D - - - - - - - V L L F D E P T S A L D P E L V G E V L R I M Q Q
MalK		134	L S G G Q R Q R V A I G R T L V A - E P S - - - - - - - V F L L D E P L S N L D A A L R V Q M R I E I S R
PstB		152	L S G G Q Q Q R L C I A R G I - A I R P E - - - - - - - V L L L D E P C S A L D P I S T G R I E E L I T E
OppD1	(Ec)	165	F S G G Q C Q R I G I A R A L - I L E P K - - - - - - - L I I C D E P V S A L D V S I Q A Q V V N L L Q Q
OppD2	(Ec)	169	F S G G M R Q R V M I A M A L L - C R P K - - - - - - - L L I A D E P T T A L D V T V Q A Q I M T L L N E
OppD	(St)	167	F S G G M R Q R V M I A M A L L - C R P K - - - - - - - L L I A D E P T T A L D V T V Q A Q I M T L L N E
RbsA	(N)	144	L S I G D Q Q M V E I A K V L S F - E S K - - - - - - - V I I M D E P T D A L T D T E T E S L F R V I R E
SfuC	(Sm)	136	L S G G Q Q Q R V A L A R A L S Q Q - P R - - - - - - - L M L L D E P F S A L D T G L R A A T R K A V A E

[a] Proteins were from *E. coli* unless otherwise indicated (9). Ec, *Escherichia coli*; St, *Salmonella typhimurium*; N, amino-terminal half of the polypeptide; Sm, *Serratia marcescens*.

amino acid 167) is homologous to ENVARSLGKSP at residue 428. Comparison with sequences in GenBank revealed no larger homologies to other proteins.

The SfuB protein was not found on polyacrylamide gels loaded with [^{35}S]methionine-labeled minicells derived from strain DS410(pSZ1) or with strain H1443 containing the entire pSZ1 insert (pAA1) or the *Bgl*II-*Pst*I fragment (pAA9) on pT7-5 (27). Therefore, synthesis of SfuB in a coupled transcription-translation system programmed by plasmid pAA9 (*sfuB*) was studied. A protein band with an apparent molecular mass of 40 kDa was found on a gel loaded with an unheated sample (Fig. 4, lane 2) but not on a gel loaded with a heated sample (lane 1). Instead, the latter contained radioactive material at the top of the gel which was present in a much smaller quantity in the lane with the unheated sample. Very hydrophobic proteins such as SfuB tend to be insoluble upon heating in sample buffer containing sodium dodecyl sulfate and do not enter polyacrylamide gels (see references 10 and 11 and literature cited therein). The determined molecular mass was less than the calculated 57 kDa. However, it was previously found that the electrophoretic mobilities of very hydrophobic proteins vary with different electrophoretic conditions (10, 11). It was assumed that the protein band seen only in the cell-free system when programmed with a plasmid containing *sfuB* was in fact the SfuB protein.

(iii) SfuC protein. The amino acid sequence deduced from the nucleotide sequence is composed of 345 residues (Fig. 1). It shows strong homologies to the nucleotide-binding proteins of PBP-dependent transport systems (9) (Table 1) which have previously been observed for the FhuC (2, 4), FecE (23), and BtuD (7) proteins of the ferrichrome, iron-citrate, and vitamin B_{12} transport sytems. The GKS/T and DEP sequences are completely conserved, and the flanking regions are largely homologous to the other proteins. These proteins, including SfuC, are rather hydrophilic but are found in the membrane fraction and are thought to be bound to the cytoplasmic side of the cytoplasmic membrane. Indeed, SfuC was found in the membrane fraction of cells containing *sfuA*, *sfuB*, and *sfuC* on pAA1 (Fig. 3, lane 2) but was absent in cells expressing only *sfuA* and *sfuB* (pAA122) (Fig. 3, lanes 6 and 7). Unfortunately, we could not observe SfuC in Triton X-100 extracts of cell envelopes that supposedly contained components of the cytoplasmic membrane. After treatment with Triton X-100–$MgCl_2$, no SfuC was left (Fig. 3, lanes 3 and 4), indicating that it was most likely degraded by cellular proteases despite the presence of added protease inhibitors. We have observed previously that the FhuC protein is also subject to degradation (11).

Conclusions. The DNA fragment of *S. marcescens* described in this paper is sufficient to enable an *aroB* mutant of *E. coli* K-12 to grow on iron-limited nutrient broth-dipyridyl medium. The three polypeptides deduced from the nucleotide sequence were identified; one localized in the periplasm, and one localized in the membrane fraction. The very hydrophobic SfuB protein is certainly also embedded in the membrane. Its molecular weight is nearly twice that of cytoplasmic membrane proteins usually found in PBP-dependent transport systems, and in this respect it resembles the FhuB transport protein (10, 11). The structures and the subcellular locations of the three Sfu proteins are characteristic for PBP-dependent transport systems which include a PBP, one or two very hydrophobic proteins, and a rather hydrophilic protein with nucleotide-binding domains in the cytoplasmic membrane. It is concluded that the three *sfu* genes determine the transport of Fe^{3+} across the cytoplasmic membrane. Moreover, the results presented above corroborate the previous finding (27) that transport of Fe^{3+} into *E. coli* via this system occurs independently of an outer membrane receptor protein and the *tonB*, *exbB*, and *exbD* gene products. Thus, the Sfu-catalyzed Fe^{3+} transport in *E. coli* is the first reported instance of a system in a member of the family *Enterobacteriaceae* in which no active transport across the outer membrane seems to be required. How and in what form Fe^{3+} traverses the outer membrane of *E. coli* and *S. marcescens* remains to be determined. Presumably, iron bound to secreted ligands of the intermediary metabolism, such as citrate, traverses the outer membrane via nonspecific pores. The periplasmic SfuA could capture the complex either as such or after loading iron onto a periplasmic ligand and channel it into the cytoplasmic membrane transport pathway.

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